Hip posture affects the firing properties of motor units in the tibialis anterior muscle

J Neurophysiol 133: 1074–1082, 2025.
First published March 5, 2025; doi:10.1152/jn.00448.2024

JNP JOURNAL OF NEUROPHYSIOLOGY

RESEARCH ARTICLE

Control of Movement

Hip posture affects the firing properties of motor units in the tibialis anterior muscle

Tetsuya Hirono,[1,2] Taian M. Vieira,[3] Alberto Botter,[3] and Kohei Watanabe[2]

[1]*Human Health Sciences, Graduate School of Medicine, Kyoto University, Kyoto, Japan;* [2]*Laboratory of Neuromuscular Biomechanics, School of Health and Sport Sciences, Chukyo University, Toyota, Japan; and* [3]*Laboratorio di Ingegneria del Sistema Neuromuscolare (LISiN), Department of Electronics and Telecommunication, Politecnico di Torino, Turin, Italy*

Abstract

In this study, we contend the firing properties of motor units change due to nonphysiological sources. We specifically ask whether changes in the fibular nerve length, without a concurrent change in tibialis anterior architecture, affect motor unit firing and recruitment strategies. We tested this hypothesis based on high-density surface electromyograms (EMGs) collected from the tibialis anterior of 18 healthy young adults for two hip postures, flexed and extended. To control for changes in peripheral nerve length, conduction time between electrical stimulation and generation of compound action potentials in extensor digitorum brevis was measured for the two hip postures during rest. Motor units were decomposed from EMGs obtained during sustained isometric dorsiflexion at 10% of the maximal voluntary contraction (MVC), and during ramp isometric contractions up to 20% MVC. Individual motor unit firings were identified and tracked between the two postures. Nerve conduction time was significantly shorter in hip flexed than in hip extended posture ($P < 0.01$), suggesting that peripheral nerve was stretched in the flexed hip posture. MVC torque was not different between flexed and extended postures ($P = 0.254$). Motor unit firing rates during sustained contraction at 10% of MVC, and during ramp-up contraction to 20% of MVC were significantly lower during flexed hip posture than during extended hip posture ($P < 0.05$). Hip flexion posture, which likely result in a stretching of the fibular nerve, was observed to reduce the average firing rate of active motor units during relatively low contractions.

NEW & NOTEWORTHY Peripheral nerve condition can affect motor unit activations. Sciatic and fibular nerves are stretched by ankle dorsiflexion, knee extension, and hip flexion. Hip flexion posture, which likely result in a stretching of the fibular nerve, was observed to reduce the average firing rate of active motor units during relatively low contraction. Proximal joint posture, which does not directly influence muscle architecture, should be considered to interpret neural input properties.

decomposition; fibular nerve; high-density surface electromyography; nerve conduction velocity; peroneal nerve

INTRODUCTION

Peripheral nerves are slacked and stretched with joint movement. When excessively stretched, just like muscles, the peripheral nerve mechanical property evaluated using shear wave velocity could change (1–3), which may limit the joint range of motion. Maximum ankle dorsiflexion joint range of motion with hip flexion and knee extension is less than that with hip extension and knee extension, due to feeling discomfort (4). Because the degree of gastrocnemius and soleus stretching is not influenced by hip joint, the nociceptive afferents related to the stiffness of other tissues including sciatic nerve also restricts range of motion. Since hip joint flexion can pull the sciatic nerve (5), the main contributors are considered as peripheral nerves including sciatic, tibial, and common fibular nerves. Peripheral nerves are subjected to length changes with joint movement, just as muscles, being therefore independent contributors to limit joint range of motion (6).

Mechanical and neural properties of peripheral nerves and muscles may change with pathological conditions and with changes in joint angle (7, 8). In clinical situations, peripheral neuropathy was reported to affect motor unit firing patterns and thus motor performance. For example, patients with

Correspondence: T. Hirono (hirono.tetsuya.4r@kyoto-u.ac.jp).
Submitted 1 October 2024 / Revised 25 October 2024 / Accepted 27 February 2025

Charcot-Marie-Tooth disease type 1A have low motor unit firing rate (9). Patients with diabetes mellitus also exhibit low motor unit firing rate (10) and greater motor unit firing variability (11). Patients with peripheral neuropathy, such as carpal tunnel syndrome (12), ulnar neuropathy (13), and diabetes mellitus (14), have also stiffer peripheral nerves. The degree of nerve slackness, which may also result from changes in joint angle, has been observed to affect conduction time measurements (15, 16). For example, when moving the hip joint from extension to flexion, while keeping the knee joint extended, conduction time measured by stimulating the common fibular nerve was observed to decrease (16). The sciatic and the fibular nerves are pulled proximally as the hip flexes, decreasing the degree of nerve slackness. Thus, given the stimulation site was fixed, distally on the fibular nerve, the distance traveled by action potentials elicited at such location until reaching the extensor digitorum brevis muscle and then the detection electrodes was expected to be shorter in the hip flexed posture. Another study, using ultrasound shear wave elastography, further reported that shear wave velocities of sciatic and tibial nerves were all greater with hip flexion and knee extension than with hip and knee extension (1). Collectively, these observations indicate that changes in joint angle affect the degree of nerve slackness, increasing or decreasing the conduction time from the spinal cord to the muscles. A nociceptor exists on the peripheral nerve with branches from the nerve itself (17–19). The nociceptor could be sensitive to its stretching and the afferent feedback might modulate the descending neural strategy.

The purpose of the present study was to investigate whether proximal joint angle affects the firing pattern of motor units in the tibialis anterior muscle. We manipulated hip joint angle while keeping constant ankle and knee joint angles. Notwithstanding the literature supporting a decrease in fibular nerve slackness when flexing the hip (16), we aimed to control for changes in nerve slackness indirectly in this study. We measured conduction time from fibular head to extensor digitorum brevis for the two hip postures. The present study focused on motor units of tibialis anterior because, being it a dorsiflexor muscle, changes in hip posture would be expected to affect only the fibular nerve and not the studied muscle. Our hypotheses were that the firing properties of motor units in tibialis anterior during submaximal dorsiflexion would change with changes in peripheral nerve stretching, resulting from changes in hip joint flexion.

METHODS

Participants

Eighteen healthy young adults (4 women) participated in the present study. The mean and standard deviation values for age, height, and body mass were 30.3 ± 6.0 yr, 175.9 ± 7.1 cm, and 68.8 ± 11.3 kg, respectively. None of them reported physical problems such as, vestibular disease, neurological dysfunctions, and musculoskeletal lesions. The purpose and procedures were explained to the participants before they provided informed written consent to participate in the study, which was conducted in accordance with the Declaration of Helsinki and approved by the local Ethics Committee (R3944).

Experimental Protocol

The present study is a cross-sectional design. The overview of the experimental protocol is shown in Fig. 1. Two postures were considered; extended hip joint posture (Ext), where peripheral nerve would not be stretched, and flexed hip joint posture (Flex), where peripheral nerve would be stretched. In Ext posture, subjects were leaning against a backrest, inclined at 30° raised from chair seat. In Flex posture, they kept their hip flexed as much as possible not to feel any uncomfortable pain. The hip flexion angle was measured by a goniometer. Under the Ext or Flex postures, the procedures were performed on the same day, in random order.

After performing several submaximal contractions of dorsiflexion and maximal contractions as familiarization, subjects performed two maximum voluntary contractions (MVC) of dorsiflexion, with 2-min rest in between (Biodex system 4; Biodex Medical Systems, Inc., Shirley, NY). The highest torque value defined the MVC torque, used to scale submaximal isometric: *1*) sustained contraction at 10% of MVC torque for 120 s; *2*) ramp-up contraction to 20% MVC, consisting of 10-s ascending phase, 20-s hold phase, and 10-s descending phase. The sustained and ramp contractions were performed after familiarization.

Peripheral Nerve Conduction Time

To confirm peripheral nerve stretching, an evaluation of nerve conduction time of deep fibular nerve was performed. To detect the difference in conduction time between hip postures, the conduction time from fibular head (stimulation site) to extensor digitorum brevis [electromyogram (EMG) detection site] was evaluated. We adopted this configuration to maximize the nerve pathway and therefore the conduction time differences between conditions. A dry electrode array was used to estimate the direction of extensor digitorum brevis fascicles (20). A linear array of eight monopolar electrodes (ELSCH008, OT Bioelettronica, Turin, Italy) was attached over the muscle belly of extensor digitorum brevis, and the reference electrode was placed over the fifth metatarsophalangeal joint to calculate the nerve conduction time. A second reference electrode was attached over the lateral malleolus (WS2; OT Bioelettronica, Turin, Italy). Stimulation places were below the fibular head, along the fibular nerve path (16). A constant-current neuromuscular stimulator (Model DS7AH; Digitimer, Welwyn Garden City, UK) delivering a monophasic rectangular pulse with a duration of 200 μs was used. A small cathode electrode (1 × 1 cm) was positioned ~1 cm distally with respect to the fibular head, along the fibular nerve path, with three large anodes (3.5 × 4.5 cm) placed over and around the patella. The stimulation of the fibular nerve can lead to the activation of both tibialis anterior and extensor digitorum brevis muscles (21). We recorded the latencies from the electrical stimulation to the generation of the action potential of extensor digitorum brevis while participants were relaxed in both postures. Surface electromyograms (EMGs) were acquired in monopolar derivation with a multichannel amplifier (EMG-Quattrocento; OT Bioelettronica, Turin, Italy): 180 V/V amplification factor, 10,240 Hz sampling rate, 16-bit A/D converter. Single-differential EMGs were computed and the innervation zone was identified (22). Nerve

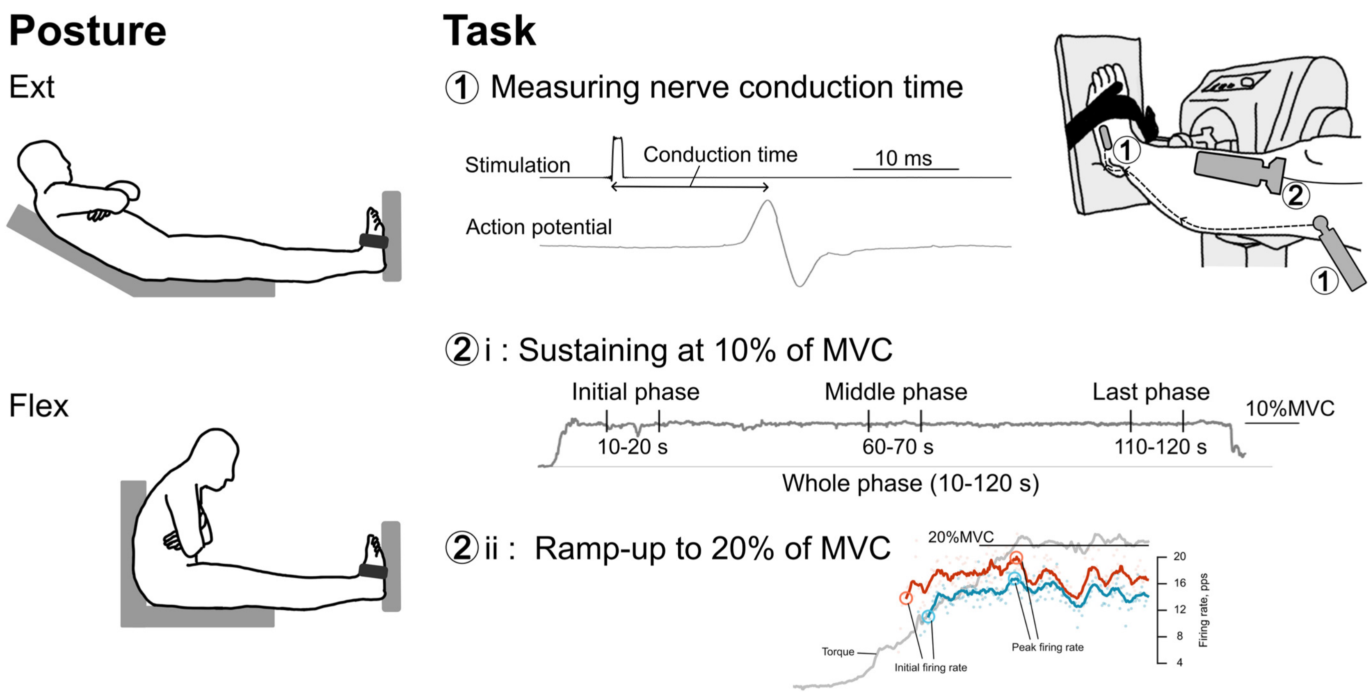

Figure 1. Overview of the study and experimental setup. Participants sat in two postures; extended hip joint (Ext) and flexed hip joint (Flex) postures. Their left foot was fixed on an attachment of Biodex during the experiment. First, electro stimulation was applied at posterior fibular head and an actional potential of extensor digitorum brevis was recorded to calculate the peripheral nerve conduction time, which could estimate peroneal nerve stretching (1). Following measurements of maximum voluntary contraction (MVC) torque, sustained isometric contraction of dorsiflexion at 10% of MVC for 120 s and ramp-up contraction with 10-s increasing phase (2i) and 10-s ramp-up contraction to 20% of MVC (2ii) were performed in each hip position. The red and blue circles represent individual motor unit firing rate and red and blue lines represent smoothed firing rates.

conduction time was computed as the time from when the current pulse was generated, rising edge of the trigger pulse commanding the stimulation device, to the instant of the positive peak of the action potential detected by the channel closest to the innervation zone of the extensor digitorum brevis (Fig. 1).

High-Density Surface Electromyography

During voluntary dorsiflexion tasks, high-density surface EMG signals were recorded from the tibialis anterior using a semidisposable adhesive grid of 64 electrodes (GR08MM1305, OT Bioelettronica, Turin, Italy). A disposable bi-adhesive form (KIT08MM1305, OT Bioelettronica) including cavities in correspondence of the grid's electrodes was used to attach the electrodes to the skin. The foam's cavities were filled with conductive paste (Ten20, Weaver and Co., Aurora, CO) to establish the electrical contact between the skin and the electrodes. After cleaning the skin with abrasive paste (Nuprep, D. O. Weaver & Co, Aurora, CO) and water (23), the grid of electrodes was placed 1 cm lateral from the edge of the tibia and over the belly of the tibialis anterior muscle (24). A reference electrode was attached to the tibial tuberosity. During submaximal contractions, high-density surface EMG signals were obtained using the same EMG amplifier reported earlier, sampled at a rate of 2,048 Hz. Torque signal from the dynamometer machine (Biodex system 4; Biodex Medical Systems, Inc., Shirley, NY) was sampled synchronously with the EMGs, through the auxiliary inputs of the multichannel EMG amplifier. The exerted and target torque levels were displayed on a monitor in real time as visual feedback.

Recorded monopolar EMGs were decomposed into single motor units with a validated algorithm (DEMUSE software, v.6.1; The University of Maribor, Slovenia; 25, 26), running in MATLAB (R2021a, MathWorks GK, Tokyo, Japan). After the signals were differentiated between adjacent electrodes in the longitudinal direction, 59-channel differentiated signals were decomposed and individual motor units were identified. Any physiologically irregular motor unit firing (less than 4 and over 30 Hz) (27–30) and individual motor unit with firing rates showing a coefficient of variation of over 30% were discarded (31). All the decomposition results were manually inspected in each task. In addition, detected motor units were tracked between two postures using the convolution kernel compensation (CKC) technique in DEMUSE software to calculate the motor unit filters that were estimated at one condition and the same filters were transferred to the other condition (32). In 10% of MVC sustained task, motor unit firing rates were calculated as four intervals between 10 s and 20 s, between 60 s and 70 s, between 100 s and 110 s, and between 10 s and 110 s after the start of contraction, as the initial, middle, last, and whole phase (Fig. 1, *2i*). Moreover, interspike interval variability in the same phases was calculated. All firings within each interval were used to calculate mean firing rate and interspike interval variability. In ramp-up to 20% of MVC task, recruitment threshold of each motor unit was defined as the torque level where the initial firing was identified. To evaluate the initial firing rate and peak firing rate during the ramp-up phase, moving average of firing rate was calculated with a window of 10 firings, afterward initial firing rate and peak firing rate were picked up (Fig. 1, *2ii*).

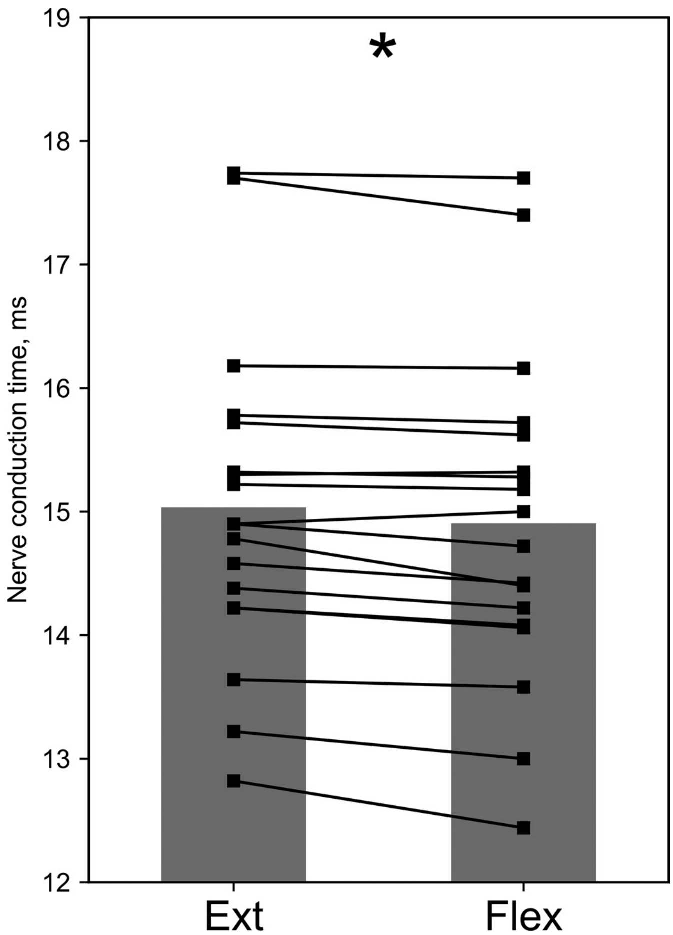

Figure 2. Peripheral nerve conduction time. Each plot and line represent individual data. The bar represents the mean values in each position. *A significant difference between extended (Ext) and flexed (Flex) hip joint postures ($P < 0.05$).

Statistical Analysis

All statistical analyses were performed using a statistical software (SPSS v.29.0, IBM Japan Inc., Tokyo, Japan). The Gaussian distribution of all variables was checked using Shapiro–Wilk test. Paired *t* tests were performed to compare MVC torque and nerve conduction time between Ext and Flex postures. Because the multilevel model statistical analysis is recommended (33, 34), for comparisons between detected motor units in each posture (nontracked motor units), linear mixed model analyses of variance, with subjects as a random factor and posture as a fixed factor, and with unstructured variance-covariance matrix, were performed. For comparisons between tracked motor units between two postures, linear mixed model analyses of variance, with motor unit as a random factor and posture as a fixed factor, and with unstructured variance-covariance matrix, were performed. The mean differences (Δ) and 95% confidence interval (95% CI) were calculated. To examine the relationship between the amount of fibular nerve stretching and tracked motor unit firing properties, the changes in the variables from Ext to Flex posture were calculated and Spearman's correlation coefficient were calculated. Significance was set as $P < 0.05$.

RESULTS

Joint Angles, Peripheral Nerve Conduction Times, and MVC Torque

In Ext and Flex postures, hip flexion angle was 32.5 ± 4.3° and 82.2 ± 14.6°, respectively (0° = hip and knee fully extended, supine position; 90° = trunk perpendicular to the femur). The paired *t* test was performed and found that the nerve conduction time was significantly shorter in Flex posture (14.9 ± 1.4 ms) than in Ext posture (15.0 ± 1.3 ms) (Δ 0.13 ms; 95% CI 0.06–0.19 ms; $P < 0.01$) (Fig. 2). The paired *t* test was performed and found that MVC torques were not significantly different between Ext (23.0 ± 1.7 N·m) and Flex postures (24.1 ± 1.3 N·m, $P = 0.254$).

Motor Unit Firing Behaviors during Sustained Contraction at 10% of MVC

The number of detected motor units was 276 and 265 in Ext and Flex postures, respectively. The number of tracked motor units was 201, and 11.2 per participant. Figure 3 shows the differences in motor unit firing rate in each phase in tracked motor units (Fig. 3, *top*) and nontracked motor units (Fig. 3, *bottom*). During all phases, motor unit firing rates in Flex posture were significantly lower than those in Ext posture, considering both tracked (whole phase: Δ 0.27 pps, 95% CI 0.17–0.48 pps, $P < 0.01$; initial phase: Δ 0.25 pps, 95% CI 0.10–0.40 pps, $P < 0.01$; middle phase: Δ 0.28 pps, 95% CI 0.14–0.43 pps, $P < 0.01$; last phase: Δ 0.26 pps, 95% CI 0.14–0.39 pps, $P < 0.01$) and nontracked motor units (whole phase: Δ 0.28 pps, 95% CI 0.05–0.50 pps, $P = 0.018$; initial phase: Δ 0.35 pps, 95% CI 0.04–0.66 pps, $P = 0.026$; middle phase: Δ 0.29 pps, 95% CI 0.05–0.53 pps, $P = 0.019$; last phase: Δ 0.26 pps, 95% CI 0.03–0.48 pps, $P = 0.026$). Figure 4 shows the differences in interspike interval variabilities in each phase in tracked motor units (Fig. 4, *top*) and nontracked motor units (Fig. 4, *bottom*). In both tracked or nontracked motor units, the variability during middle phases were significantly smaller in Flex than in Ext posture (tracked motor units: Δ 1.08%, 95% CI 0.64%–1.53%, $P < 0.01$; nontracked motor units: Δ 1.00%, 95% CI 0.36%–1.66%, $P < 0.01$), but during the last phase, there were no significances in either tracked (Δ −0.16%; 95% CI −0.56 to 0.25%; $P = 0.449$) or nontracked motor units (Δ 0.67%; 95% CI −0.09% to 1.42%; $P = 0.084$). The variability in only tracked motor units during initial phase and whole phase was significantly smaller in Flex than in Ext posture (initial phase: Δ 0.58%, 95% CI 0.22%–0.95%, $P = 0.049$; whole phase: Δ 0.58%, 95% CI 0.22%–0.95%, $P < 0.01$), but not in nontracked motor units (initial phase: Δ 0.85%, 95% CI −0.12% to 1.82%, $P = 0.085$; whole phase: Δ 0.62%, 95% CI −0.87% to 1.32%, $P = 0.086$).

Motor Unit Firing Behaviors during Ramp-Up to 20% of MVC

The number of detected motor units was 226 and 225 in Ext and Flex postures, respectively. The number of tracked motor units was 209, and 11.6 per participant. Figure 5 shows the differences in recruitment threshold in tracked motor units (Fig. 5, *left*) and nontracked motor units (Fig. 5, *right*). Recruitment thresholds in Flex posture were significantly higher than those in Ext posture in tracked motor unit comparison (Δ 2.0%MVC; 95% CI 1.6–2.5%MVC; $P < 0.01$), but not in nontracked comparison (Δ 0.8%MVC; 95% CI −0.3 to 1.9%

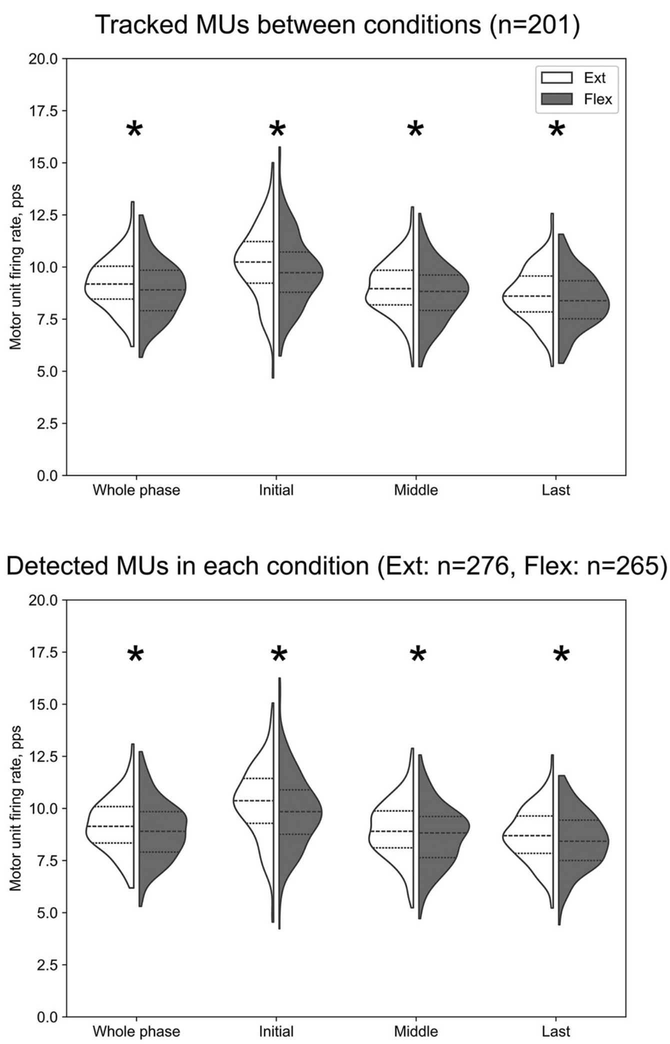

Figure 3. Motor unit firing rate during sustained contraction at 10% of maximum voluntary contraction. The lines in each violin plot show the quartiles of the data. *Top* shows motor unit firing rate in tracked motor units, and *bottom* shows those in detected motor units in each position. Whole phase was an interval between 10 s and 110 s after the beginning of sustained contraction. Initial, middle, and last phases were defined as the 10-s interval with the starts of 10 s, 60 s, and 100 s after the beginning of sustained contraction. MU, motor unit. *A significant difference between extended (Ext) and flexed (Flex) hip joint postures ($P < 0.05$).

MVC; $P = 0.167$). Figure 6 shows initial motor unit firing rate (Fig. 6, *left*) and peak firing rate (Fig. 6, *right*) in tracked motor units (Fig. 6, *top*) and nontracked motor units (*Fig. 6*, *bottom*). In tracked motor units, initial and peak motor unit firing rates were significantly lower in Flex than those in Ext posture (initial: Δ 0.39 pps, 95% CI 0.17–0.60 pps, $P < 0.01$; peak: Δ 0.54 pps, 95% CI 0.37–0.72 pps, $P < 0.01$). Similarly, in nontracked motor units, initial and peak firing rates were significantly lower in Flex than in Ext posture (initial: Δ 0.52 pps, 95% CI 0.14–0.90 pps, $P < 0.01$; peak: 0.71 pps, 95% CI 0.25–1.17 pps, $P < 0.01$).

Relationship between the Change in Fibular Nerve Conduction Time and Tracked Motor Unit Firing Properties

There were no significant correlations between the change in nerve conduction time and motor unit firing rate during sustained task at 10% of MVC (whole phase, $P = 0.602$; initial phase, $P = 0.745$; middle phase, $P = 0.356$; last phase, $P = 0.713$), and motor unit recruitment threshold during ramp-up task to 20% of MVC ($P = 0.363$), and firing rate during ramp-up task (initial firing rate; $P = 0.277$, peak firing rate; $P = 0.115$).

DISCUSSION

The present study investigated the effects of proximal joint posture on motor unit firing behaviors of tibialis anterior during low-intensity voluntary dorsiflexion. During Flex posture where the slackness of peripheral nerve was removed, motor unit firing rate was significantly lower. However, there was not any relationships between the amount of peripheral nerve slackness and motor unit firing activations. This is the first study to reveal the differences in motor unit firing behavior

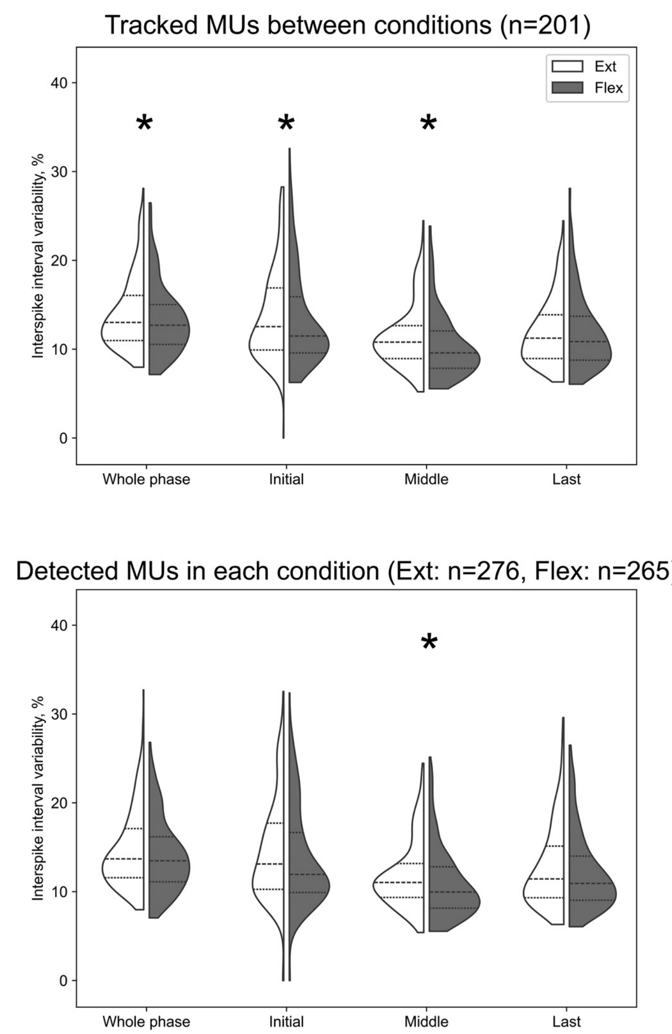

Figure 4. Interspike interval variability during sustained contraction at 10% of maximum voluntary contraction. The lines in each violin plot show the quartiles of the data. *Top* shows interspike interval variability in tracked motor units, and *bottom* shows those in detected motor units in each position. Whole phase was an interval between 10 s and 110 s after the beginning of sustained contraction. Initial, middle, and last phases were defined as the 10-s interval with the starts of 10 s, 60 s, and 100 s after the beginning of sustained contraction. MU, motor unit. *A significant difference between extended (Ext) and flexed (Flex) hip joint postures ($P < 0.05$).

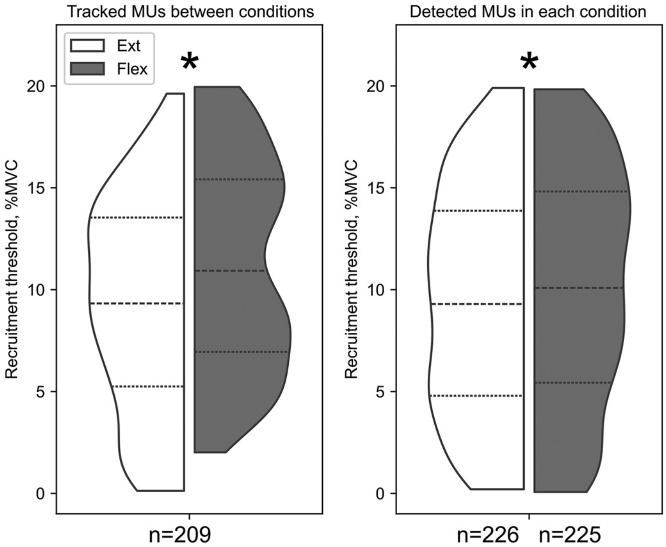

Figure 5. Recruitment threshold during ramp-up contraction to 20% of maximum voluntary contraction (MVC). The lines in each violin plot show the quartiles of the data. MU, motor unit. *A significant difference between extended (Ext) and flexed (Flex) hip joint postures ($P < 0.05$).

with different proximal joint posture, which does not directly influence muscle architecture.

To estimate the peripheral nerve length condition, we measured and compared the nerve conduction time between two different postures. In previous studies (15, 16), nerve conduction time was measured to estimate the peripheral nerve conditions. One of them examined common fibular nerve conduction time by electrical stimulation and measurement of action potential from extensor digitorum brevis (16). Recent previous studies (1, 3, 35) confirmed the sciatic and common fibular nerve stretching using shear wave elastography as similar posture as that in the present study. Robinson and Probyn (5) simulated and reported that the distance between center of femoral head and sciatic nerve was 41 mm in the supine position and the pulled sciatic nerve from 0° to 90° hip flexion was 64 mm. In our current study, the difference between two hip flexion angles was ~50°. Considering the conduction speed to be constant at ~100 mm/ms (36, 37), the 0.1 ms mean difference we observed in conduction time would correspond to ~10 mm change in the traveling distance from the fibular head to the extensor digitorum brevis. This figure is well within the 35 mm of excursion reported recently for a change in hip joint angle 50° (5). It should be noted that we computed conduction time based on M-Wave measurements taken distal to the knee joint, where the fibular nerve could be stimulated easily. Changes in hip posture would indeed be expected to account for a relatively small change in nerve length, likely explaining the 10 mm figure. Our results are therefore in agreement with the notion that hip flexion decreases the amount of fibular nerve slackness.

During sustained contraction at 10% of MVC, motor unit firing rate was significantly lower in peripheral nerve stretching position (Fig. 3) and during ramp-up contraction to 20% of MVC (Fig. 6). The lower firing rate in peripheral nerve stretching position could be partly explained by the changes in recruitment threshold of the motor units. Tracked motor units were recruited at higher torque levels during peripheral nerve stretching position than during nerve slack position (Fig. 5), suggesting that number of active motor units would be increased during peripheral nerve stretching. Focusing on tracked motor units, there were few tracked motor units in Flex posture during initial force exertion (such as from 0 to 3% of MVC in Fig. 5), suggesting that the initial force production might require other motor unit activations to compensate the inactive tracked motor unit firings. It could be possible that other additional recruited motor units might activate to produce force exertion, leading to the reduction of tracked motor unit firing rate during low-intensity force exertion. Whereas focusing on nontracked motor units, motor units with significantly higher recruitment thresholds were recruited during Flex (Fig. 5). It could also be possible that motor units with high recruitment threshold can produce high force exertion due to large size of motor unit. In general, motor units with high recruitment thresholds fires at low frequency during

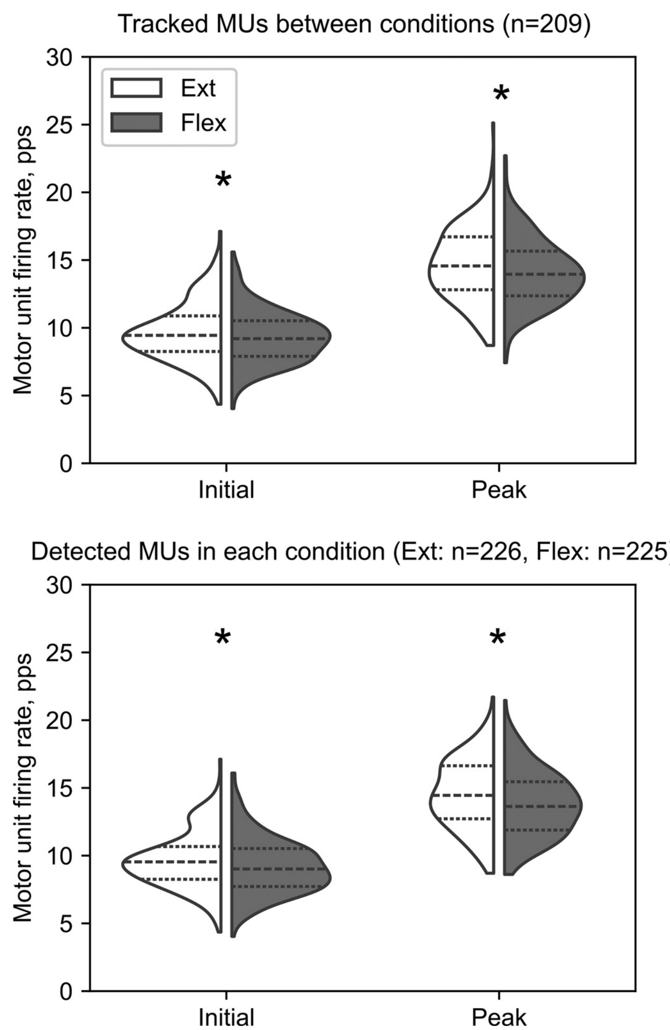

Figure 6. Motor unit firing rate during ramp-up contraction to 20% of maximum voluntary contraction. The lines in each violin plot show the quartiles of the data. *Top* shows interspike interval variability in tracked motor units, and *bottom* shows those in detected motor units in each position. MU, motor unit. *A significant difference between extended (Ext) and flexed (Flex) hip joint postures ($P < 0.05$).

submaximal constant exertion, presumably because of their relatively larger size according to the size principle (38, 39). Thus, the same intensity torque exertion would require to less firing rate of motor units in peripheral nerve stretching position.

Peripheral nerve, especially epineurium and perineurium contain a plexus of nerve fibers, which is called as nervi nervorum (19), with branches from the nerve itself (17, 18). The nervi nervorum can play as a nociceptor to be sensitive to stretching in the long axis of the peripheral nerve (40, 41). The afferent feedback from nervi nervorum to motor unit activations is not fully understood, but some literatures investigated the effect of experimental muscle pain on motor unit recruitment strategies. The previous studies have commonly reported a decrease in firing rate of lower-threshold motor units, following the excitation of higher-threshold motor units (42–45). The adaptation is believed to be related to group III and IV afferent inhibition for motor neuron pool. Although we confirmed the participants did not feel any uncomfortable pain during Flex posture, the afferent inhibition might alter motor unit recruitment strategy in response to peripheral nerve stretching. However, the present study did not directly evaluate afferent system including nociceptor or group III and IV pathway. A determinate mechanism, why our results occurred during peripheral nerve stretching, was unclear from the present study protocol, but it is certain that recruitment strategy was different with proximal joint posture.

Low-intensity torque exertion required a reduction in motor unit firing rate, such as sustained at 10% of MVC (Fig. 3). This was observed in both tracked and nontracked motor units. These results might suggest that recruitment pattern to produce low-intensity force exertion was influenced by peripheral nerve stretching, or that motor units of different size are sensitive differently to nerve stretching. Experimental muscle pain can reduce initial motor unit firing rate in a previous study, where the participants performed 4-min dorsiflexion at 25% of MVC (42). Although we confirmed that the participants did not feel uncomfortable pain during the posture, stretch-related nociceptor in peripheral nerve might play as the similar inhibition pathway that might be active to alter motor unit recruitment strategy.

Motor unit firing variability during sustained contraction at 10% of MVC was smaller in Flex than in Ext posture (Fig. 4). In patients with diabetes mellitus who have a stiff peripheral nerve (14), their motor unit firing variability was significantly greater than age-matched healthy control subjects' (11). Although the stiff peripheral nerve might be different condition from the peripheral nerve stretching, if considering that the peripheral nerve conditions were degenerated in the patients, our results suggested that the firing variability in neuropathy patients could not be influenced by stiff peripheral nerve condition, but caused by other neuromuscular mechanism.

We should note the absence of correlation between the change in nerve conduction time and the change in motor unit firing behaviors from Ext to Flex posture in the present results. Even though we could detect a difference in conduction time, this difference may not be sufficiently sensitive to the change in nerve length between the two positions. Indeed, since the stimulation site was distal, the change in the nerve length from the stimulation site to the muscle may have been too small for resulting in an appreciable change in conduction time. Moreover, our conduction time estimates were limited by the temporal resolution of our measures, as with 10 kHz sampling frequency we cannot assess differences in conduction time shorter than 0.1 ms. Therefore, the lack of correlation between conduction time and motor unit firing behavior does not exclude an effect of changes in nerve length on the motor unit firing pattern. Other competing mechanisms possibly explaining the changes in motor unit firing behavior with hip posture are associated with the neural circuits. As other possibility, reflex circuits from one muscle to the other muscles were observed in previous reports (46, 47). In the present study, the degree of stretching hamstring muscle was different between two hip positions. The difference might perhaps have modulated tibialis anterior firing behaviors. As another reason, cerebellum, brain stem, and afferent system including deep sensation could be related to the changes in motor unit firing behaviors (48). The neural mechanism other than peripheral nerve stretching should be investigated in future studies. In addition, if we could use ultrasound shear wave elastography, which is a good evaluation tool to measure the stiffness of soft tissue, we could have assessed the degree of peripheral nerve stretching in detail. This is a possible limitation of the present study.

Conclusions

The novel findings of the present study suggested that proximal joint angle could change motor unit recruitment thresholds and reduce the average firing rate of active motor units, even both tracked and nontracked motor units. These results could be of importance for neurophysiology when considering joint position and force exertion, such as treatment or training for patients or athletes with considering the conditions or adaptations of neuromuscular systems.

DATA AVAILABILITY

Data will be made available upon reasonable request.

ACKNOWLEDGMENTS

We thank Prof. Aleš Holobar of the University of Maribor, Slovenia, for supporting the analyses of motor unit firing properties using the DEMUSE tool.

GRANTS

This work was also financially supported by a Grant-in-Aid from the Japan Society for the Promotion of Science Fellows under Grant No. 24K20528.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

T.H., T.M.V., A.B., and K.W. conceived and designed research; T.H. performed experiments; T.H. analyzed data; T.H., T.M.V., A.B., and K.W. interpreted results of experiments; T.H. prepared figures; T.H. drafted manuscript; T.H., T.M.V., A.B., and K.W. edited and revised manuscript; T.H., T.M.V., A.B., and K.W. approved final version of manuscript.

REFERENCES

1. **Andrade RJ, Freitas SR, Hug F, Coppieters MW, Sierra-Silvestre E, Nordez A.** Spatial variation in mechanical properties along the sciatic and tibial nerves: an ultrasound shear wave elastography study. *J Biomech* 136: 111075, 2022. doi:10.1016/j.jbiomech.2022.111075.
2. **Andrade RJ, Lacourpaille L, Freitas SR, McNair PJ, Nordez A.** Effects of hip and head position on ankle range of motion, ankle passive torque, and passive gastrocnemius tension. *Scand J Med Sci Sports* 26: 41–47, 2016. doi:10.1111/sms.12406.
3. **Andrade RJ, Freitas SR, Hug F, Le Sant G, Lacourpaille L, Gross R, McNair P, Nordez A.** The potential role of sciatic nerve stiffness in the limitation of maximal ankle range of motion. *Sci Rep* 8: 14532, 2018. doi:10.1038/s41598-018-32873-6.
4. **Mitchell B, Bressel E, McNair PJ, Bressel ME.** Effect of pelvic, hip, and knee position on ankle joint range of motion. *Phys Ther Sport* 9: 202–208, 2008. doi:10.1016/j.ptsp.2008.08.002.
5. **Robinson LR, Probyn L.** How much sciatic nerve does hip flexion require? *Can J Neurol Sci* 46: 248–250, 2019. doi:10.1017/cjn.2018.378.
6. **Nordez A, Gross R, Andrade R, Le Sant G, Freitas S, Ellis R, McNair PJ, Hug F.** Non-muscular structures can limit the maximal joint range of motion during stretching. *Sports Med* 47: 1925–1929, 2017. doi:10.1007/s40279-017-0703-5.
7. **Ginanneschi F, Dominici F, Biasella A, Gelli F, Rossi A.** Changes in corticomotor excitability of forearm muscles in relation to static shoulder positions. *Brain Res* 1073–1074: 332–338, 2006. doi:10.1016/j.brainres.2005.12.021.
8. **Hoffmann G, Kamper DG, Kahn JH, Rymer WZ, Schmit BD.** Modulation of stretch reflexes of the finger flexors by sensory feedback from the proximal upper limb poststroke. *J Neurophysiol* 102: 1420–1429, 2009. doi:10.1152/jn.90950.2008.
9. **Noto YI, Watanabe K, Holobar A, Kitaoji T, Tsuji Y, Kojima Y, Kitani-Morii F, Mizuno T, Nakagawa M.** High-density surface electromyography to assess motor unit firing rate in Charcot-Marie-Tooth disease type 1A patients. *Clin Neurophysiol* 132: 812–818, 2021. doi:10.1016/j.clinph.2020.11.040.
10. **Favretto MA, Andreis FR, Cossul S, Negro F, Oliveira AS, Marques JLB.** Differences in motor unit behavior during isometric contractions in patients with diabetic peripheral neuropathy at various disease severities. *J Electromyogr Kinesiol* 68: 102725, 2023. doi:10.1016/j.jelekin.2022.102725.
11. **Watanabe K, Gazzoni M, Holobar A, Miyamoto T, Fukuda K, Merletti R, Moritani T.** Motor unit firing pattern of vastus lateralis muscle in type 2 diabetes mellitus patients. *Muscle Nerve* 48: 806–813, 2013. doi:10.1002/mus.23828.
12. **Kantarci F, Ustabasioglu FE, Delil S, Olgun DC, Korkmazer B, Dikici AS, Tutar O, Nalbantoglu M, Uzun N, Mihmanli I.** Median nerve stiffness measurement by shear wave elastography: a potential sonographic method in the diagnosis of carpal tunnel syndrome. *Eur Radiol* 24: 434–440, 2014. doi:10.1007/s00330-013-3023-7.
13. **Paluch L, Noszczyk B, Nitek Z, Walecki J, Osiak K, Pietruski P.** Shear-wave elastography: a new potential method to diagnose ulnar neuropathy at the elbow. *Eur Radiol* 28: 4932–4939, 2018. doi:10.1007/s00330-018-5517-9.
14. **Dikici AS, Ustabasioglu FE, Delil S, Nalbantoglu M, Korkmaz B, Bakan S, Kula O, Uzun N, Mihmanli I, Kantarci F.** Evaluation of the tibial nerve with shear-wave elastography: a potential sonographic method for the diagnosis of diabetic peripheral neuropathy. *Radiology* 282: 494–501, 2017. doi:10.1148/radiol.2016160135.
15. **Hsu K, Robinson LR.** Effect of shoulder and elbow position on ulnar nerve conduction. *Muscle Nerve* 60: 88–90, 2019. doi:10.1002/mus.26489.
16. **Broadhurst PK, Robinson LR.** Effect of hip and knee position on nerve conduction in the common fibular nerve. *Muscle Nerve* 56: 519–521, 2017. doi:10.1002/mus.25585.
17. **Bove GM, Light AR.** Calcitonin gene-related peptide and peripherin immunoreactivity in nerve sheaths. *Somatosens Mot Res* 12: 49–57, 1995. doi:10.3109/08990229509063141.
18. **Marshall J.** Bradshaw lecture on nerve-stretching for the relief or cure of pain. *Br Med J* 2: 1173–1179, 1883. doi:10.1136/bmj.2.1198.1173.
19. **Bove GM, Light AR.** The nervi nervorum. *Pain Forum* 6: 181–190, 1997. doi:10.1016/S1082-3174(97)70011-4.
20. **Lapatki BG, van Dijk JP, van de Warrenburg BP, Zwarts MJ.** Botulinum toxin has an increased effect when targeted toward the muscle's endplate zone: a high-density surface EMG guided study. *Clin Neurophysiol* 122: 1611–1616, 2011. doi:10.1016/j.clinph.2010.11.018.
21. **Carbonaro M, Seynnes O, Maffiuletti NA, Busso C, Minetto MA, Botter A.** Architectural changes in superficial and deep compartments of the tibialis anterior during electrical stimulation over different sites. *IEEE Trans Neural Syst Rehabil Eng* 28: 2557–2565, 2020. doi:10.1109/TNSRE.2020.3027037.
22. **Masuda T, Sadoyama T.** The propagation of single motor unit action potentials detected by a surface electrode array. *Electroencephalogr Clin Neurophysiol* 63: 590–598, 1986. doi:10.1016/0013-4694(86)90146-x.
23. **Merletti R, Cerone GL.** Tutorial. Surface EMG detection, conditioning and pre-processing: best practices. *J Electromyogr Kinesiol* 54: 102440, 2020. doi:10.1016/j.jelekin.2020.102440.
24. **Mani D, Almuklass AM, Hamilton LD, Vieira TM, Botter A, Enoka RM.** Motor unit activity, force steadiness, and perceived fatigability are correlated with mobility in older adults. *J Neurophysiol* 120: 1988–1997, 2018. doi:10.1152/jn.00192.2018.
25. **Farina D, Holobar A, Merletti R, Enoka RM.** Decoding the neural drive to muscles from the surface electromyogram. *Clin Neurophysiol* 121: 1616–1623, 2010. doi:10.1016/j.clinph.2009.10.040.
26. **Holobar A, Farina D, Gazzoni M, Merletti R, Zazula D.** Estimating motor unit discharge patterns from high-density surface electromyogram. *Clin Neurophysiol* 120: 551–562, 2009. doi:10.1016/j.clinph.2008.10.160.
27. **Adam A, De Luca CJ.** Firing rates of motor units in human vastus lateralis muscle during fatiguing isometric contractions. *J Appl Physiol (1985)* 99: 268–280, 2005. doi:10.1152/japplphysiol.01344.2004.
28. **Welsh SJ, Dinenno DV, Tracy BL.** Variability of quadriceps femoris motor neuron discharge and muscle force in human aging. *Exp Brain Res* 179: 219–233, 2007. doi:10.1007/s00221-006-0785-z.
29. **Kirk EA, Christie AD, Knight CA, Rice CL.** Motor unit firing rates during constant isometric contraction: establishing and comparing an age-related pattern among muscles. *J Appl Physiol (1985)* 130: 1903–1914, 2021. doi:10.1152/japplphysiol.01047.2020.
30. **Kamen G, Knight CA.** Training-related adaptations in motor unit discharge rate in young and older adults. *J Gerontol A Biol Sci Med Sci* 59: 1334–1338, 2004. doi:10.1093/gerona/59.12.1334.
31. **Fuglevand AJ, Winter DA, Patla AE.** Models of recruitment and rate coding organization in motor-unit pools. *J Neurophysiol* 70: 2470–2488, 1993. doi:10.1152/jn.1993.70.6.2470.
32. **Francic A, Holobar A.** On the reuse of motor unit filters in high density surface electromyograms recorded at different contraction levels. *IEEE Access* 9: 115227–115236, 2021. doi:10.1109/ACCESS.2021.3104762.
33. **Tenan MS, Marti CN, Griffin L.** Motor unit discharge rate is correlated within individuals: a case for multilevel model statistical analysis. *J Electromyogr Kinesiol* 24: 917–922, 2014. doi:10.1016/j.jelekin.2014.08.014.
34. **Guo Y, Jones EJ, Inns TB, Ely IA, Stashuk DW, Wilkinson DJ, Smith K, Piasecki J, Phillips BE, Atherton PJ, Piasecki M.** Neuromuscular recruitment strategies of the vastus lateralis according to sex. *Acta Physiol (Oxf)* 235: e13803, 2022. doi:10.1111/apha.13803.
35. **Andrade RJ, Freitas SR, Hug F, Le Sant G, Lacourpaille L, Gross R, Quillard JB, McNair PJ, Nordez A.** Chronic effects of muscle and nerve-directed stretching on tissue mechanics. *J Appl Physiol (1985)* 129: 1011–1023, 2020. doi:10.1152/japplphysiol.00239.2019.
36. **Falck B, Stålberg E.** Motor nerve conduction studies: measurement principles and interpretation of findings. *J Clin Neurophysiol* 12: 254–279, 1995.
37. **Hursh JB.** Conduction velocity and diameter of nerve fibers. *Am J Physiol Legacy Content* 127: 131–139, 1939. doi:10.1152/ajplegacy.1939.127.1.131.
38. **Henneman E, Somjen G, Carpenter DO.** Functional significance of cell size in spinal motoneurons. *J Neurophysiol* 28: 560–580, 1965. doi:10.1152/jn.1965.28.3.560.

39. **Erim Z**, **De Luca CJ**, **Mineo K**, **Aoki T.** Rank-ordered regulation of motor units. *Muscle Nerve* 19: 563–573, 1996. doi:10.1002/(SICI)1097-4598(199605)19:5<563::AID-MUS3>3.0.CO;2-9.
40. **Sugar O.** Victor Horsley, John Marshall, nerve stretching, and the nervi nervorum. *Surg Neurol* 34: 184–187, 1990. doi:10.1016/0090-3019(90)90071-v.
41. **Sauer SK**, **Bove GM**, **Averbeck B**, **Reeh PW.** Rat peripheral nerve components release calcitonin gene-related peptide and prostaglandin E2 in response to noxious stimuli: evidence that nervi nervorum are nociceptors. *Neuroscience* 92: 319–325, 1999. doi:10.1016/s0306-4522(98)00731-3.
42. **Farina D**, **Arendt-Nielsen L**, **Graven-Nielsen T.** Experimental muscle pain reduces initial motor unit discharge rates during sustained submaximal contractions. *J Appl Physiol (1985)* 98: 999–1005, 2005. doi:10.1152/japplphysiol.01059.2004.
43. **Martinez-Valdes E**, **Negro F**, **Farina D**, **Falla D.** Divergent response of low- versus high-threshold motor units to experimental muscle pain. *J Physiol* 598: 2093–2108, 2020. doi:10.1113/JP279225.
44. **Martinez-Valdes E**, **Negro F**, **Arvanitidis M**, **Farina D**, **Falla D.** Pain-induced changes in motor unit discharge depend on recruitment threshold and contraction speed. *J Appl Physiol (1985)* 131: 1260–1271, 2021. doi:10.1152/japplphysiol.01011.2020.
45. **Tucker K**, **Butler J**, **Graven-Nielsen T**, **Riek S**, **Hodges P.** Motor unit recruitment strategies are altered during deep-tissue pain. *J Neurosci* 29: 10820–10826, 2009. doi:10.1523/JNEUROSCI.5211-08.2009.
46. **Masugi Y**, **Obata H**, **Inoue D**, **Kawashima N**, **Nakazawa K.** Neural effects of muscle stretching on the spinal reflexes in multiple lower-limb muscles. *PLoS One* 12: e0180275, 2017. doi:10.1371/journal.pone.0180275.
47. **Marchand-Pauvert V**, **Nielsen JB.** Modulation of heteronymous reflexes from ankle dorsiflexors to hamstring muscles during human walking. *Exp Brain Res* 142: 402–408, 2002. doi:10.1007/s00221-001-0942-3.
48. **Debenham MIB**, **Smuin JN**, **Grantham TDA**, **Ainslie PN**, **Dalton BH.** Hypoxia and standing balance. *Eur J Appl Physiol* 121: 993–1008, 2021. doi:10.1007/s00421-020-04581-5.